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Brief Definitive Report

Epinephrine Exerts Anticoagulant Effects during Human Endotoxemia

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Summary
To determine the effect of a physiologically relevant elevation in the plasma concentrations of epinephrine on the activation of the hemostatic mechanism during endotoxemia, 17 healthy men were studied after intravenous injection of lipopolysaccharide (LPS, 2 ng/kg), while receiving a continuous infusion of epinephrine (30 ng/kg/min) started either 3 h \((n=5)\) or 24 h \((n=6)\) before LPS injection, or an infusion of normal saline \((n=6)\). Activation of the coagulation system (plasma concentrations of thrombin–antithrombin III complexes and prothrombin fragment F1+2) was significantly attenuated in the groups treated with epinephrine compared with subjects injected with LPS only \((P, 0.05)\). Epinephrine enhanced LPS-induced activation of fibrinolysis (plasma levels of tissue-type plasminogen activator and plasmin–α2–antiplasmin complexes; \(P, 0.05)\), but did not influence inhibition of fibrinolysis (plasminogen activator inhibitor type I). In subjects infused with epinephrine, the ratio of maximal activation of coagulation and maximal activation of fibrinolysis was reduced by \(>50\). Hence, epinephrine exerts antithrombotic effects during endotoxemia by concurrent inhibition of coagulation, and stimulation of fibrinolysis. Epinephrine, whether endogenously produced or administered as a component of treatment, may limit the development of disseminated intravascular coagulation during systemic infection.

A systemic inflammatory response syndrome (SIRS) is frequently associated with disseminated intravascular coagulation, a serious complication that significantly contributes to organ injury and mortality (1). Enhanced release of epinephrine is part of the early host response to acute systemic infection and inflammation (2, 3). Although the role of epinephrine in substrate and energy metabolism, and cardiovascular control during SIRS has been widely recognized (2–4), knowledge of the in vivo effects of this catecholamine on activation of the hemostatic mechanism is limited.

It is conceivable that epinephrine influences coagulant and fibrinolytic pathways during SIRS for several reasons. Epinephrine has been reported to stimulate the release of tissue-type plasminogen activator (tPA) in humans in vivo (5, 6). Further, expression of tissue factor, the essential mediator of coagulation activation during SIRS (7, 8), can be inhibited in vitro by agents that elevate cellular cAMP (9, 10). Since β-adrenergic stimulation results in an increase in cellular cAMP concentrations (11, 12), it can be anticipated that epinephrine is able to influence coagulation. Knowledge of the hemostatic effect of epinephrine is important not only for understanding the outcomes of endogenous adrenergic responses on the regulation of coagulation during SIRS, but also for the therapeutic use of catecholamines in patients with septic shock. Therefore, in the present study we sought to determine the effect of a constant intravenous epinephrine infusion on the activation of the hemostatic mechanism during low dose endotoxia in normal humans.

Materials and Methods
Study Design and Subjects. This study was performed simultaneously with an investigation determining the effect of epineph-
Epinephrine on LPS-induced cytokine release (13). The study was approved by the Institutional Review Board, and written informed consent was obtained from all subjects before enrollment in the study. Nineteen male subjects, aged 28 ± 1 (mean ± SE) yr, were admitted to the Adult Clinical Research Center of the New York Hospital-Cornell University Medical Center for 4 d (day 0 to day 3). On day 1, 8 subjects were started on a constant intravenous infusion of epinephrine (30 ng/kg/min; Parke-Davis, Morris Plains, NJ), starting at 9:00 AM. On day 2, at 9:00 AM, 6 of these 8 subjects were intravenously injected with LPS (2 ng/kg) at 9:00 AM. Epinephrine was freshly provided by Dr. H.D. Hochstein (Bureau of Biologics, Food and Drug Administration, Bethesda, MD) at a dose of 2 ng/kg body weight (EPI-24 group). The infusion of epinephrine was continued until 6 h after LPS administration (3:00 PM). The other 11 subjects were randomized on day 2 to receive either a constant intravenous infusion of epinephrine (30 ng/kg/min; Parke-Davis, Morris Plains, NJ), starting at 6:00 AM (EPI-3 group), or an equivalent volume of normal saline (LPS group). On day 2, at 9:00 AM, 6 of these 8 subjects were intravenously injected with LPS (2 ng/kg) at 9:00 AM. Epinephrine was freshly provided by Dr. H.D. Hochstein (Bureau of Biologics, Food and Drug Administration, Bethesda, MD) at a dose of 2 ng/kg body weight (EPI-24 group). The infusion of epinephrine was continued until 6 h after LPS administration (3:00 PM). The other 11 subjects were randomized on day 2 to receive either a constant intravenous infusion of epinephrine (30 ng/kg/min; n = 5), starting at 6:00 AM (3 h before LPS administration) and continued until 3:00 PM (EPI-3 group), or an equivalent volume of normal saline (n = 6) (LPS group). These subjects also were injected with LPS (2 ng/kg) at 9:00 AM. Epinephrine was freshly prepared in normal saline every 8 h. 3 h before the administration of LPS a radial arterial catheter was placed in all subjects for blood sampling.

Sampling and assays. On day 1, venous blood was obtained before the start of infusion with epinephrine (t = 0 h), and at 1, 2, 4, and 8 h thereafter. On day 2, arterial blood was obtained at 6:00 AM (i.e., directly before the start of the infusion of epinephrine in the EPI-3 group or the start of saline infusion in the LPS group) (t = −3 h), directly before the injection of LPS (t = 0 h), and 0.5, 1, 1.5, 2, 3, 4, 5, 6, 8, 12, and 24 h thereafter. All blood samples were centrifuged at 4°C for 20 min at 1,600 g and plasma was stored at −7°C until assayed.

Activation of the coagulation system was determined in citrated plasma by measurements of prothrombin fragment F1+2 and thrombin–antithrombin III (TAT) complexes (ELISAs; Behringwerke AG, Marburg, Germany). Activation of fibrinolysis was determined in citrated plasma by measurements of tPA antigen and plasminogen activator inhibitor type 1 (PAI-1) antigen (ELISAs; Innogenetics, Nijmegen, The Netherlands), PAI-1 activity by amidolytic assay (Chromogenix, Mölndal, Sweden), and plasmin–α2-antiplasmin (PAP) complexes (8). Soluble E-selectin levels were measured in K3-EDTA anticoagulated plasma by ELISA (14).

Statistical Analysis. All values are given as means ± SEM. Differences within groups were analyzed by one-way analysis of variance. Differences between groups were analyzed by two-way analysis of variance (interaction treatment and time). Two sample comparisons were performed by Wilcoxon test. P < 0.05 was considered to represent a statistically significant difference.

Results

A diminution of coagulation. Infusion of epinephrine only (day 1) did not influence the plasma concentrations of F1+2 or TAT complexes (data not shown). Injection of LPS induced transient rises in plasma F1+2 and TAT complexes, peaking after 4 h (4.61 ± 1.03 nmol/l and 31.9 ± 4.7 ng/ml, respectively; both P < 0.05 versus time). Both EPI-3 and EPI-24 strongly attenuated LPS-induced coagulation activation (Fig. 1). In the EPI-3 group, peak concentrations of F1+2 and TAT complexes were 1.88 ± 0.34 nmol/l and 16.5 ± 5.6 ng/ml, respectively, in the EPI-24 group, 2.25 ±

Table 1. Effect of Epinephrine on Fibrinolysis

<table>
<thead>
<tr>
<th>Time</th>
<th>tPA antigen</th>
<th>PAP complexes</th>
<th>PAI-1 antigen</th>
<th>PAI-1 activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>h</td>
<td>ng/ml</td>
<td>nmol/l</td>
<td>ng/ml</td>
<td>ng/ml</td>
</tr>
<tr>
<td>0</td>
<td>3.5 ± 0.6</td>
<td>5.9 ± 1.1</td>
<td>7.1 ± 1.0</td>
<td>4.0 ± 0.8</td>
</tr>
<tr>
<td>1</td>
<td>11.6 ± 1.8</td>
<td>14.4 ± 2.3</td>
<td>7.3 ± 0.8</td>
<td>4.4 ± 0.9</td>
</tr>
<tr>
<td>2</td>
<td>6.1 ± 1.7</td>
<td>9.9 ± 0.9</td>
<td>8.6 ± 0.6</td>
<td>4.9 ± 0.8</td>
</tr>
<tr>
<td>4</td>
<td>4.6 ± 1.3</td>
<td>8.1 ± 0.8</td>
<td>5.6 ± 0.8</td>
<td>5.0 ± 0.8</td>
</tr>
<tr>
<td>8</td>
<td>3.3 ± 0.6</td>
<td>7.5 ± 1.0</td>
<td>7.9 ± 0.9</td>
<td>7.1 ± 1.0</td>
</tr>
<tr>
<td>21</td>
<td>3.6 ± 0.6</td>
<td>6.3 ± 1.1</td>
<td>10.6 ± 1.3</td>
<td>4.5 ± 0.7</td>
</tr>
<tr>
<td>24</td>
<td>3.2 ± 0.6</td>
<td>6.1 ± 0.9</td>
<td>10.6 ± 1.4</td>
<td>5.3 ± 0.5</td>
</tr>
<tr>
<td>P vs. time</td>
<td>P &lt; 0.05</td>
<td>P &lt; 0.05</td>
<td>P &lt; 0.05</td>
<td>NS</td>
</tr>
</tbody>
</table>

Data are mean ± SE of eight subjects receiving a continuous 24-h intravenous infusion of epinephrine (30 ng/kg/min) starting at t = 0. NS, nonsignificant.
0.55 nmol/l and 14.6 ± 4.4 ng/ml, respectively (all P < 0.05 versus LPS only).

Activation of Fibrinolysis. Infusion of epinephrine only (day 1) modestly stimulated fibrinolysis, as reflected by transient rises in the plasma concentrations of tPA and PAP complexes. Epinephrine also caused a relatively late increase in the plasma levels of PAI-1 antigen (Table 1).

Administration of LPS resulted in a marked activation of fibrinolysis, followed by a strong inhibition coinciding with maximal coagulation activation (Fig. 2). Peak levels of indexes of fibrinolytic activation were found after 1.5 to 2 h, i.e., before maximal coagulation activation: tPA antigen (32.1 ± 2.0 ng/ml) and PAP complexes (33.9 ± 4.5 nmol/l) (both P < 0.05 versus time). Peak levels of indexes of inhibition of fibrinolysis were found thereafter, i.e., after 4 h: PAI-1 antigen (140.7 ± 9.9 ng/ml) and PAI activity (42.0 ± 3.2 ng/ml) (both P < 0.05 versus time). Both EPI-3 and EPI-24 enhanced LPS-induced activation of fibrinolysis (Fig. 2). The EPI-3 group showed peak levels of tPA antigen and PAP complexes were 47.8 ± 2.6 ng/ml and 50.2 ± 5.4 nmol/l (both P < 0.05 versus LPS only), respectively. In the EPI-24 group, these levels were 44.2 ± 2.7 ng/ml and 50.6 ± 7.3 nmol/l (both P < 0.05 versus LPS only), respectively. By contrast, neither EPI-3 nor EPI-24 influenced inhibition of fibrinolysis, as reflected by unchanged increases in the plasma levels of PAI-1 antigen and PAI activity in the three study groups (Fig. 2).

Ratio of Coagulation and Fibrinolysis. Since epinephrine appeared to inhibit coagulation and enhance fibrinolysis, we were interested in the effect of epinephrine on the balance between coagulation and fibrinolysis. For this purpose we calculated the ratio of the peak plasma levels of TAT complexes and PAP complexes. Both EPI-3 and EPI-24 reduced this ratio to <50% of the ratio found after administration of LPS only (P < 0.05; Table 2).

Table 2. The Effect of Epinephrine on the Coagulation/Fibrinolysis Balance

<table>
<thead>
<tr>
<th>Subject</th>
<th>LPS</th>
<th>EPI-3</th>
<th>EPI-24</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.61</td>
<td>0.69</td>
<td>0.54</td>
</tr>
<tr>
<td>2</td>
<td>0.83</td>
<td>0.49</td>
<td>0.51</td>
</tr>
<tr>
<td>3</td>
<td>1.17</td>
<td>0.18</td>
<td>0.14</td>
</tr>
<tr>
<td>4</td>
<td>0.89</td>
<td>0.42</td>
<td>0.64</td>
</tr>
<tr>
<td>5</td>
<td>1.07</td>
<td>0.38</td>
<td>0.29</td>
</tr>
<tr>
<td>6</td>
<td>1.08</td>
<td>-</td>
<td>0.25</td>
</tr>
<tr>
<td>Mean ± SE</td>
<td>0.94 ± 0.08</td>
<td>0.43 ± 0.08*</td>
<td>0.40 ± 0.08*</td>
</tr>
</tbody>
</table>

Data are ratios of peak plasma levels of TAT complexes and PAP complexes, reflecting activation of coagulation and fibrinolysis respectively. LPS, subjects injected with LPS only; EPI-3, subjects infused with epinephrine (30 ng/kg/min) from t = −3 h to 6 h; EPI-24, subjects infused with epinephrine (30 ng/kg/min) from t = −24 h to 6 h. *P < 0.05 versus LPS.
and EPI-24 reduced LPS-induced increases in soluble E-selectin levels with epinephrine (30 ng/kg/min) from jected with LPS only (Fig. 3). Soluble E-selectin levels were not influenced by infusion of epinephrine only (day 1) (data not shown).

Discussion

This study demonstrates for the first time that epinephrine exerts a strong anticoagulant effect during endotoxemia. Epinephrine attenuated activation of the coagulation system induced by intravenous injection of LPS, and consequently enhanced activation of the fibrinolytic system. The anticoagulant properties of epinephrine were further demonstrated by a >50% reduction in the ratio of maximal coagulation activation and maximal fibrinolytic activation. These effects of epinephrine on the hemostatic mechanism were found at physiologically relevant plasma concentrations (1,000–1,200 pg/ml) (13). Indeed, the infusion of epinephrine sought to mimic two clinically relevant situations, i.e., epinephrine concentrations achieved were in the same range as those found in patients with SIRS, and the rate and dose at which epinephrine was infused resembled the rate and dose at which the hormone is started as part of supportive treatment of SIRS (13).

We chose to start the infusion of epinephrine at 3 or 24 h before LPS administration because in vitro studies had suggested that the effect of epinephrine on intracellular cAMP levels is dependent on the duration of exposure of cells to this hormone (12). Thus, incubation of mononuclear cells with epinephrine for 2 h increased cellular cAMP concentrations, while incubation for 24 h was associated with a decrease in cAMP levels (12). cAMP plays a pivotal role in many inflammatory reactions, including expression of tissue factor on mononuclear cells (9, 10), a process mediating activation of the common pathway of the coagulation system during SIRS (1, 7, 8). We hypothesized that β-adrenergic stimulation would inhibit LPS-induced tissue factor expression as a consequence of a direct increase in cAMP levels. Considering the biphasic effect of epinephrine on cellular cAMP concentrations in vitro (12), we considered it important to determine the influence of epinephrine infusions of various durations on the coagulation system. However, both EPI-3 and EPI-24 inhibited LPS-induced coagulation activation. The release of TNF, a cytokine of which the production is negatively regulated by cAMP (12, 15), was also reduced in both the EPI-3 and EPI-24 groups (13), albeit to a lesser extent in the latter group. However, the circulating levels of IL-10, the production of which is positively regulated by cAMP (16), were increased only in the EPI-3 group (13). Hence, the duration of the effect of epinephrine may vary from response to response, i.e., brief (IL-10), intermediate (TNF), or long (coagulation). We did not directly measure cAMP concentrations within circulating mononuclear cells. It should be noted, however, that we were previously unable to demonstrate enhanced tissue factor expression on circulating monocytes in this model of low grade endotoxemia (17), suggesting that cells not present in the circulation are involved in initiation of coagulation. Nonetheless, our data do not firmly establish the duration of the in vivo effect of epinephrine on intracellular cAMP, or the mechanism underlying the variable duration of epinephrine effects considered to be mediated by elevated cAMP levels.

It can be argued that decreased TNF and increased IL-10 concentrations (13) might have contributed to the anticoagulant effect of epinephrine, since TNF stimulates and IL-10 inhibits tissue factor expression in vitro (18, 19). However, it is unlikely that the inhibition of coagulation activation by epinephrine was mediated via an effect on the cytokine system. Indeed, TNF is not necessary for activation of coagulation during low grade endotoxemia (20), and IL-10 levels were unaltered in the EPI-24 group, whereas this group showed a similar inhibition of coagulation as the EPI-3 group (13). Further, neither epinephrine infusion influenced LPS-induced release of IL-6 (13), a cytokine that appears to play an important role in LPS-induced coagulation activation (21).

Continuous infusion of epinephrine per se (day 1) was associated with a transient activation of the fibrinolytic system. Earlier studies demonstrated tPA release after short-term exposure of humans to epinephrine (5, 6), which likely reflects a direct effect of epinephrine on the vascular endothelium (22). Additionally, epinephrine potentiates the release of tPA in response to injection of LPS. It should be noted that epinephrine did not cause a general endothelial cell activation, since the release of soluble E-selectin, a molecule that is shed by the vascular endothelium upon activation (14, 23), was inhibited in the EPI-3 and EPI-24 groups. Epinephrine did not influence LPS-induced release of the main inhibitor of plasminogen activation, PAI-1. The gradual increase in PAI-1 antigen during infusion of epinephrine only (day 1) likely reflects a modest acute phase protein response induced by this hormone, considering that...
PAI-1 behaves as an acute phase protein during inflammation (24), and that other acute phase proteins also gradually increased during epinephrine infusion (data not shown).

Systemic infection and acute injury lead to the activation of multiple host mediator systems. We demonstrate here that a physiologically relevant elevation in the plasma levels of epinephrine markedly influences the activation of hemo-

References


dent mechanism during endotoxemia in humans. The anticoagulant effect of epinephrine represents a property of this catecholamine not previously recognized that may be important for the understanding of the complex agonistic and antagonistic interactions between various mediator sys-
tems involved in the pathogenesis of disseminated intravas-
cular coagulation in acutely ill patients.

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